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Review on LAMP and its utilizing for the diagnosis of haemoprotozoan diseases in livestock and companion animals

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Abstract
Haemoprotozoan infections, caused by protozoan parasites such as *Trypanosoma*, *Theileria*, *Babesia*, and *Hepatozoon* species, are of significant veterinary importance due to their economic impact on livestock and companion animals. Accurate and rapid diagnosis is critical for effective disease management and control. Traditional diagnostic methods, including microscopy and polymerase chain reaction (PCR), have limitations such as low sensitivity, time consumption, requirement for skilled personnel, and the need for sophisticated laboratory infrastructure. Loop-Mediated Isothermal Amplification (LAMP) has emerged as a highly sensitive, specific, rapid, and cost-effective molecular diagnostic tool. LAMP amplifies target DNA under isothermal conditions using Bst DNA polymerase and a set of specifically designed primers, enabling visual detection through colorimetric dyes, turbidity, or fluorescence without the need for expensive equipment. This technique has been successfully applied for the detection of *Trypanosoma evansi*, *Theileria annulata*, *Babesia* spp., *Babesia gibsoni*, and *Hepatozoon canis* in field samples, demonstrating higher sensitivity and specificity compared to conventional methods. LAMP offers a simple, field-deployable, and reliable diagnostic approach suitable for early detection, epidemiological studies, and disease control programs, particularly in resource-limited settings. Its rapidity, ease of use, and adaptability position LAMP as a promising tool for improving animal health and productivity.

Keywords: LAMP, haemoprotozoan diseases, *Trypanosoma*, *Theileria*, *Babesia*, *Hepatozoon*, molecular diagnosis, livestock, companion animals, rapid detection

Introduction
Protozoan parasites are important pathogens responsible for severe infections in animals and humans globally, primarily transmitted through arthropod vectors or blood transfusion. In livestock, hemoprotozoan infections such as trypanosomosis, theileriosis, babesiosis, and anaplasmosis, caused by *Trypanosoma*, *Theileria*, *Babesia*, and *Anaplasma* species, lead to significant health problems including fever, anemia, weight loss, reduced productivity, abortion, and even death, resulting in substantial economic losses. Conventional diagnostic approaches, such as microscopic examination of blood smears, are simple and cost-effective but have limited sensitivity, particularly in detecting early or chronic infections. Advanced methods, including ELISA, *in vitro* culture, animal inoculation, and PCR, provide higher sensitivity and specificity; however, they are often expensive, technically complex, and time-consuming. PCR, for instance, requires repeated thermal cycling through denaturation, annealing, and extension steps, generating millions of DNA copies, but demands sophisticated equipment and skilled personnel, limiting its utility in field conditions. To overcome these limitations, Loop-Mediated Isothermal Amplification (LAMP) has emerged as a rapid, highly sensitive, and specific molecular technique capable of amplifying target DNA under constant temperature conditions. LAMP offers a cost-effective, simple, and field-adaptable diagnostic solution, making it highly suitable for early detection and management of hemoprotozoan infections in livestock and other animals.
The World Health Organization (WHO) recommends that an ideal diagnostic test, especially for developing and resource-limited countries, should be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free (or requiring only minimal equipment), and deliverable to the end user. In the case of haemoprotozoan infections in livestock,

conventional diagnostic methods, while effective, often face significant limitations, including high costs, longer processing times, and the need for sophisticated laboratory infrastructure. To address these challenges, Loop-Mediated Isothermal Amplification (LAMP) has emerged as a highly suitable diagnostic tool. LAMP is simple, rapid, highly sensitive and specific, cost-effective, and can be performed with minimal equipment, making it particularly valuable for on-site or field-based detection of haemoprotozoan parasites in livestock.Loop-Mediated Isothermal Amplification (LAMP)

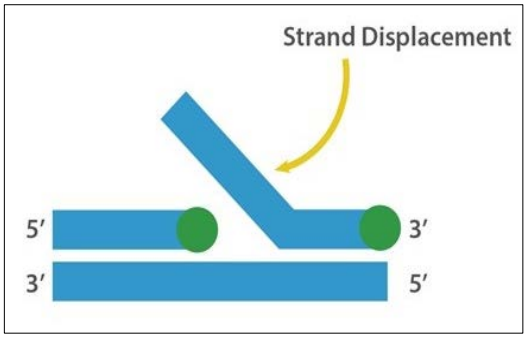
Notomi *et al*. (2000) [29] successfully developed a novel isothermal amplification technique known as LAMP, which can amplify a small number of DNA copies into millions within approximately one hour. This method offers high sensitivity and specificity and is particularly advantageous in resource-limited settings.

What is Lamp?

Loop-Mediated Isothermal Amplification (LAMP)

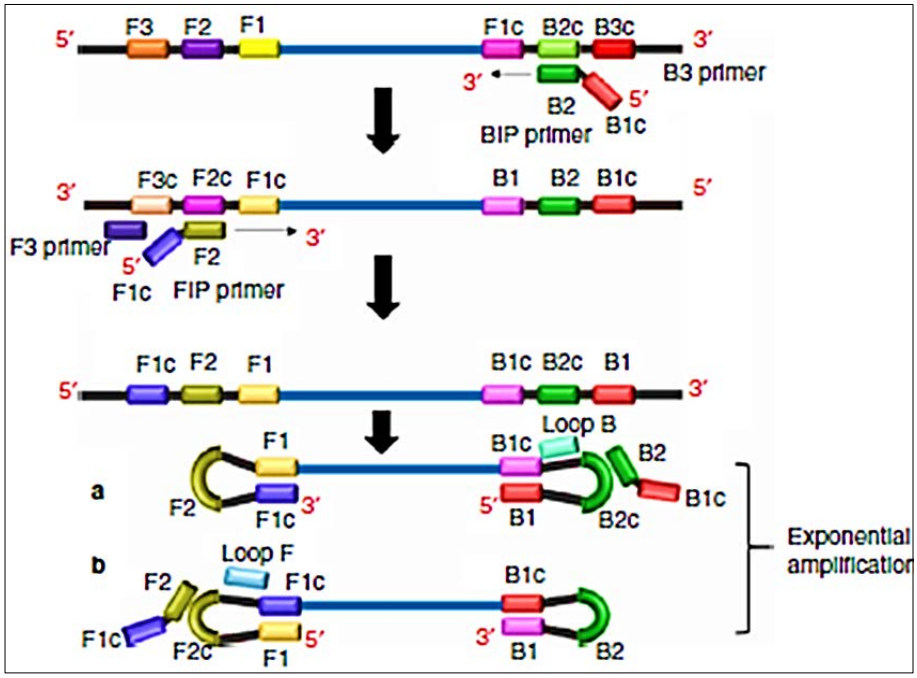
LAMP is a single-tube molecular technique that amplifies DNA under constant (isothermal) temperature conditions with high specificity, efficiency, and speed. It uses a strand-displacing DNA polymerase such as Bst polymerase along with specially designed primers that target specific DNA regions. Due to its simplicity, low cost, and rapid results, LAMP is a reliable alternative for detecting various infectious diseases, particularly in resource-limited settings.

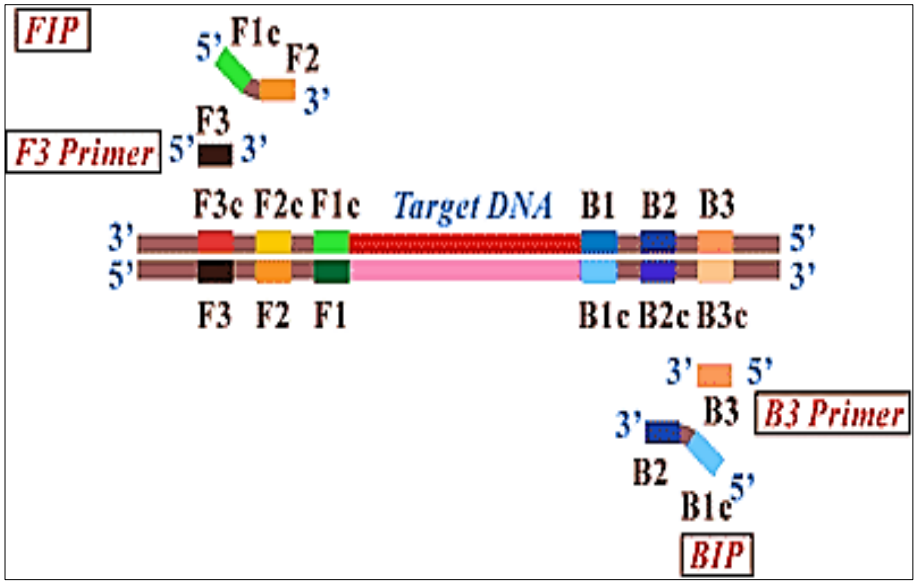
Bst DNA polymerase: It exhibits 5'→3' DNA polymerase activity with strong strand-displacement capability, enabling DNA synthesis at a constant temperature without the need for thermal cycling. The enzyme efficiently amplifies high GC-content DNA and is not hindered by complex secondary structures. Produced with high purity, it is widely used in applications requiring strand-displacement activity and supports rapid sequencing using only nanogram levels of DNA template.Strand-Displacement Activity, Refers to the ability of an enzyme to displace downstream DNA strands during synthesis. Bst DNA Polymerase (Large Fragment) is an excellent strand-displacing enzyme and remains active at elevated temperatures around 65 °C, making it ideal for LAMP reactions.

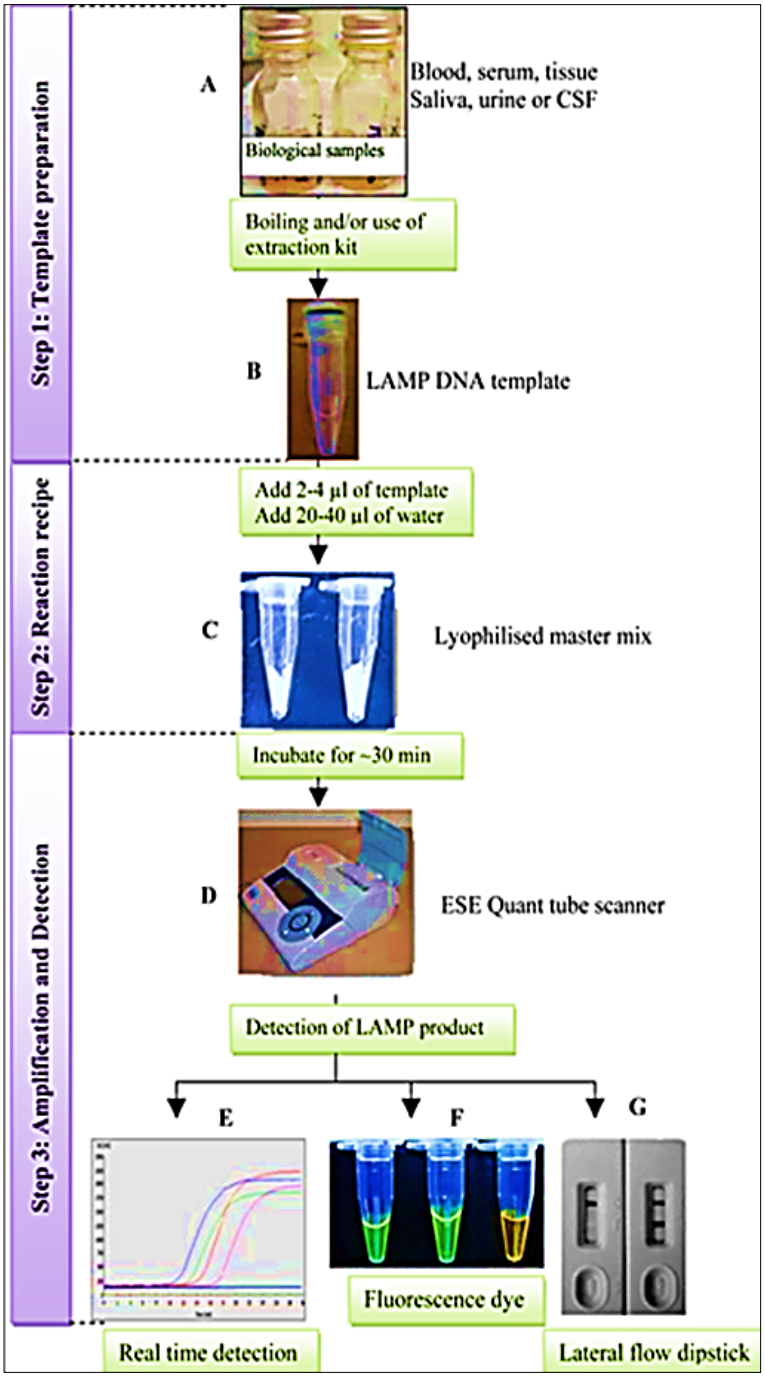


Procedure of LAMP

The Loop-Mediated Isothermal Amplification (LAMP) technique begins with the use of four to six specifically designed primers that recognize distinct regions of the target gene, including F3c, F2c, F1c on the 3′ end and B1, B2, B3 on the 5′ end. The primers include the Forward Inner Primer (FIP), Backward Inner Primer (BIP), and the outer primers F3 and B3. After collecting the blood sample, DNA is extracted using either a commercial kit or a simple lysis method. The LAMP reaction mixture typically contains dNTPs, Bst DNA polymerase, fluorescent dye for detection, primers, and the DNA template. The amplification process begins when the FIP binds to its complementary sequence on the target DNA. Bst polymerase then extends the primer and simultaneously displaces the existing DNA strand. This is followed by binding of the outer primer (F3), which initiates synthesis and displaces the newly synthesized strand, forming a loop structure due to complementary sequences at the ends. At the constant reaction temperature, continuous cycles of annealing, extension, and strand displacement occur, gradually producing a dumbbell-shaped DNA structure that serves as the starting point for exponential amplification. This structure provides multiple sites for further synthesis by inner primers, resulting in the rapid formation of large concatemeric DNA products containing multiple loop regions, characteristic of successful LAMP amplification.




FIP
F1c
F2
3'
5'
F3
F3 Primer
5'
3'
F3c F2c F1c
Target DNA
B1 B2 B3
3'
5'
5'
3'
F3 F2 F1
B1c B2c B3c
3'
5'
3'
B3
B3 Primer
B2
B1c
5'
BIP


Step 1: Template preparation
A
Blood, serum, tissue
Saliva, urine or CSF
Biological samples
Boiling and/or use of extraction kit
B
LAMP DNA template
Step 2: Reaction recipe
Add 2-4 µl of template
Add 20-40 µl of water
C
Lyophilised master mix
Incubate for ~30 min
Step 3: Amplification and Detection
D
ESE Quant tube scanner
Detection of LAMP product
E
F
G
Fluorescence dye
Real time detection
Lateral flow dipstick

LAMP Reaction Mechanism

1. Initiation: The F2 region of FIP hybridizes to the F2c region of the target DNA, initiating complementary strand synthesis.
2. The F3 primer then binds to the F3c region of the template and extends, displacing the FIP-linked complementary strand.
3. The displaced single-stranded DNA forms a loop at the 5′ end.
4. This single-stranded DNA with a 5′ loop serves as a template for BIP, where the B2 region hybridizes to the B2c region of the template.
5. DNA synthesis by BIP creates a complementary strand, opening the 5′ loop.
6. Subsequently, B3 primer binds to the B3c region and extends, displacing the BIP-linked complementary strand, forming a dumbbell-shaped DNA structure.
7. Bst DNA polymerase adds nucleotides to the 3′ end of F1, extending and opening the 5′ loop.
8. The dumbbell-shaped DNA is converted into a stem-loop structure, which serves as the initiator for the second stage of LAMP (cycling).
9. Loop primers can be added to enhance the exponential amplification of the target DNA.
10. The final LAMP products are a mixture of stem-loop DNA of varying lengths and cauliflower-like structures with multiple loops.

Amplification: Incubate the reaction mixture in a water bath at 60-65 °C for 30-60 minutes. Amplification occurs at a constant temperature using a polymerase with high strand-displacement activity.

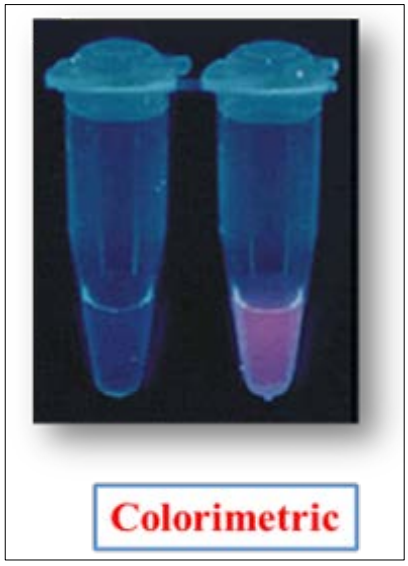
Detection of Amplified Products: The amplified LAMP products can be detected visually using nucleic acid-binding dyes such as SYBR Green, which produce a distinct color change observable to the naked eye. For more accurate and quantitative analysis, the reaction can also be monitored using simple instruments such as fluorescence readers or turbidimeters, allowing precise measurement of amplification results.

Mg^{2+}-Based Detection in LAMP

In LAMP assays, calcein dye, a metal ion-binding fluorophore, is added prior to isothermal incubation. During the reaction, calcein interacts with manganese ions (Mn^{2+}) to form an insoluble manganese-pyrophosphate complex, which generates initial fluorescence. As the LAMP reaction proceeds, free calcein binds to magnesium ions (Mg^{2+}) present in the reaction mixture, further enhancing fluorescence, which can be easily observed under UV light (365 nm). Additionally, the reaction between Mg^{2+} and calcein produces a color change from orange to green, allowing visual detection with the naked eye. A positive LAMP reaction is indicated by a green color, while a negative reaction remains orange. This calcein-based endpoint detection is widely used due to its high sensitivity and clear visual readout. The reaction can also be monitored by observing the white precipitate of magnesium pyrophosphate ($Mg_2P_2O_7$), a byproduct of the LAMP reaction, by measuring optical density at 650 nm at regular intervals.

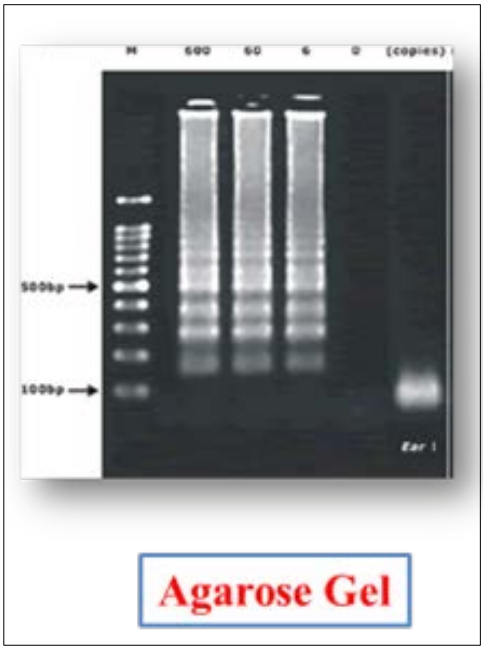
Colorimetric Detection in LAMP

SYBR Green I is a commonly used dye for colorimetric detection in LAMP assays. When added to tubes containing LAMP products, it produces a visible color change from reddish-orange to yellowish-green and exhibits fluorescence under UV light. While SYBR Green I offers good sensitivity, it can inhibit the LAMP reaction if added prior to isothermal incubation. Conversely, adding the dye post-amplification requires opening the reaction tubes, which increases the risk of false-positive results due to aerosol contamination.



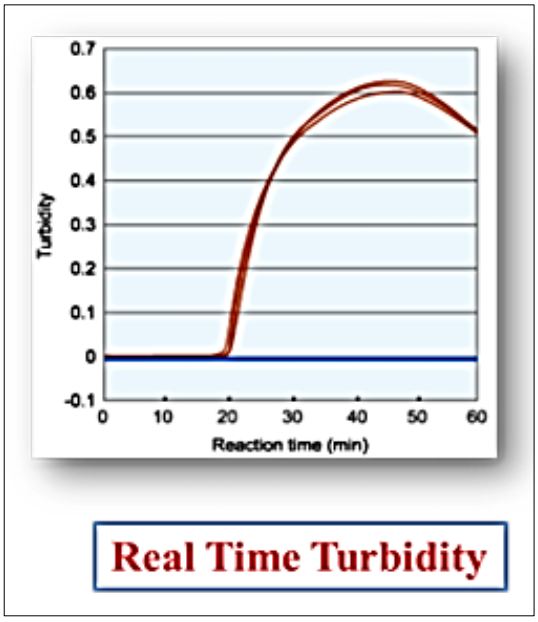
Agarose Gel Electrophoresis for LAMP Detection

Agarose gel electrophoresis can be used to visualize LAMP products. The amplified DNA forms cauliflower-like structures with multiple loops, which appear as a ladder-like pattern on the gel. Although this method effectively demonstrates the presence of LAMP products, it requires opening the reaction tubes, which increases the risk of carryover contamination. Additionally, the use of ethidium bromide, a common DNA stain, limits the application of this technique due to its highly hazardous and carcinogenic nature.



Real-Time Turbidity Detection in LAMP

Real-time monitoring of LAMP amplification can be performed using turbidity-based instruments, such as the Loopamp Realtime Turbidimeter (LA-200, LA-320, LA-500; Eiken Chemical Co., Tokyo, Japan). During the reaction, the formation of magnesium pyrophosphate precipitate causes an increase in turbidity, which can be measured in real time. A rising turbidity curve indicates a positive LAMP amplification (e.g., for Salmonella detection), whereas the absence of a curve signifies a negative result. This method allows continuous, real-time monitoring of the amplification process without the need to open reaction tubes, thereby reducing the risk of contamination (Mashooq *et al*., 2016) [25].



Sensitivity and Specificity of LAMP

The LAMP assay has been shown to be 10-100 times more sensitive than conventional PCR, with a detection limit ranging from 0.01 to 10 Pfu (Parida *et al*., 2005) [32]. It is capable of detecting very low copy numbers of DNA, making it highly suitable for samples with minimal genetic material (Notomi *et al*., 2000) [29]. LAMP can even amplify forensic samples containing DNA at femtogram levels, demonstrating its remarkable sensitivity (Dhama *et al*., 2014) [10]. The high accuracy and specificity of the LAMP reaction is largely attributed to the design of its primers, which recognize multiple distinct regions on the target gene (Nagamine *et al*., 2002) [27].

Taq DNA polymerase vs. Bst DNA Polymerase

Taq DNA polymerase is derived from the thermophilic bacterium *Thermus aquaticus* and is highly thermostable. It exhibits deoxynucleotidyl transferase activity and is commonly used for routine PCR amplification of DNA fragments up to 5 kb, as well as DNA labeling applications. In contrast, Bst DNA polymerase is derived from *Bacillus stearothermophilus* and is characterized by its strong strand displacement activity, enabling DNA synthesis at a constant temperature. This property makes Bst polymerase ideal for isothermal amplification methods, such as LAMP, and for DNA sequencing through regions with high GC content.

Differences between PCR and LAMP

Polymerase Chain Reaction (PCR) is a cyclic amplification method that requires a thermocycler. It involves repeated steps of denaturation at 95 °C, annealing at 50-60 °C, and polymerization at 72 °C. Visualization of PCR-amplified DNA is usually performed using gel electrophoresis, which requires a gel documentation system. PCR typically uses two primers to target the DNA sequence.

In contrast, Loop-Mediated Isothermal Amplification (LAMP) is an isothermal amplification method performed at a constant temperature of 60-65 °C, usually in a simple water bath. LAMP amplification can be visualized directly by color change, turbidity, or gel electrophoresis, eliminating the need for sophisticated equipment. LAMP requires 4-6 primers designed to recognize multiple regions of the target DNA, providing higher specificity and rapid amplification compared to PCR.

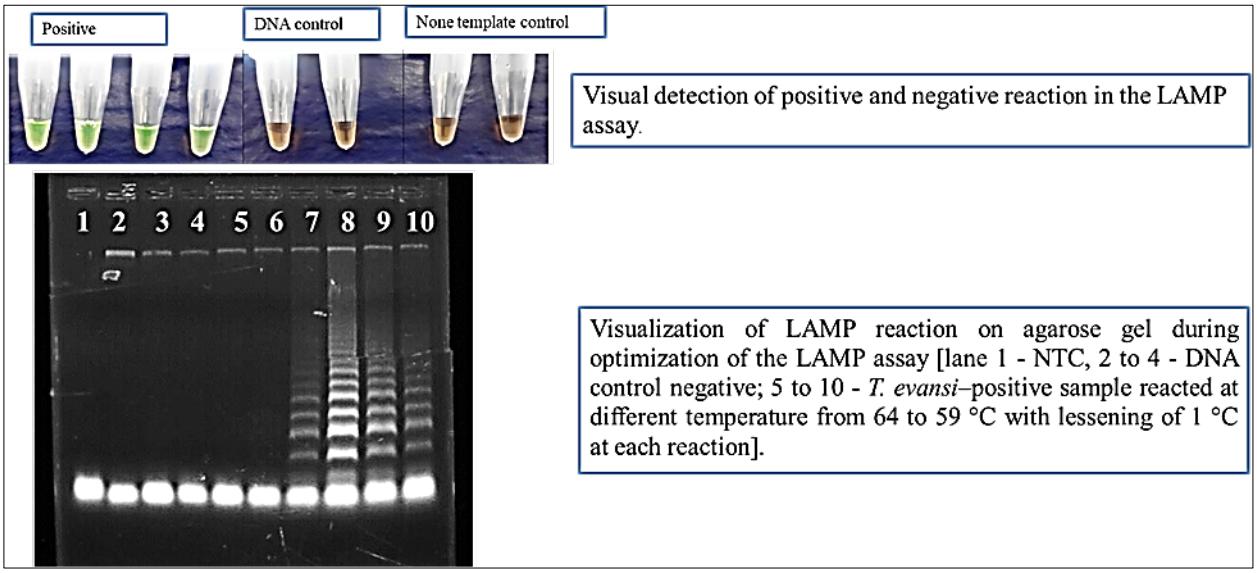
Application of LAMP in the Diagnosis of Haemoprotozoan Diseases

Trypanosoma evansi **(**Kumar *et al*., 2024**)** [21]

Animal trypanosomosis, commonly known as surra and caused by *Trypanosoma evansi*, is an economically important disease that affects a wide range of wild and domestic animals. The disease can manifest in forms ranging from acute and fatal to chronic and asymptomatic, depending on the host species. Common clinical signs include intermittent fever in chronic cases, weight loss, lethargy, anemia, enlargement of lymph nodes, and dependent edema (Desquesnes *et al*., 2002a) [9]. Traditionally, parasitological techniques such as microscopy are employed to detect trypanosomes in blood. However, these methods are limited by low sensitivity and inability to identify subclinical or carrier animals (Desquesnes *et al*., 2002a) [9]. PCR has also been used for detection, but it is time-consuming, labor-intensive, and requires skilled personnel.

To overcome these limitations, the study utilized LAMP targeting the RoTat1.2 gene, a unique gene in *T. evansi*, for rapid and sensitive detection. Blood samples were collected from 500 domestic animals suspected of hemoparasitic infections, and the results of different diagnostic methods were: microscopy 12 positive samples, RoTat1.2 PCR 33 positive samples, and LAMP assay 36 positive samples. The

LAMP assay was optimized for high sensitivity and specificity and enabled naked-eye visualization using SYBR® Green I dye, where positive samples appeared green and negative samples remained orange. The assay also produced a characteristic ladder-like pattern specific to the *T. evansi* target gene. This LAMP assay provides a rapid, simple, and reliable diagnostic tool for the detection of *T. evansi* and can be used for both diagnosis and epidemiological surveillance in regions where type A infections pose a significant threat to livestock. Its high sensitivity and specificity make it an effective tool for trypanosomosis control strategies.



Visual detection of positive and negative reaction in the LAMP assay.

Visualization of LAMP reaction on agarose gel during optimization of the LAMP assay [lane 1 - NTC, 2 to 4 - DNA control negative; 5 to 10 - *T. evansi*–positive sample reacted at different temperature from 64 to 59 °C with lessening of 1 °C at each reaction].

Theileria annulata **(**Chaouch *et al*., 2017**)** [8]

Tropical theileriosis, caused by the haemoprotozoan *Theileria annulata*, is an economically significant disease affecting cattle in tropical and subtropical regions. The parasite is transmitted by several species of ixodid ticks belonging to the *Hyalomma* genus, following their cyclical development. Tropical theileriosis can occur in various subclinical forms, impacting both milk and meat production. The present study aimed to evaluate the reliability of a LAMP-based assay targeting the cytochrome b gene, a multicopy gene of the parasite, for the detection of *T. annulata* in cattle suspected of clinical theileriosis. A total of 88 field blood samples were collected from cattle showing clinical signs of theileriosis. The diagnostic results were as follows: microscopy 80 positive samples, PCR 58 positive samples, and LAMP assay 64 positive samples. The specificity and sensitivity of the LAMP assay were compared with conventional microscopy and PCR. The results demonstrated that the LAMP assay was more sensitive than both microscopy and PCR, highlighting its potential as a rapid and reliable diagnostic tool for detecting *T. annulata* in field conditions.

Babesia bigemina* & *Babesia bovis **(**Yang *et al*., 2016**)** [44]

Babesiosis is a parasitic disease transmitted by tick vectors and is prevalent in many parts of the world, causing significant economic losses in the livestock industry (Homer, 2000; Hunfeld *et al*., 2008) [16, 17]. *Babesia bovis* and *Babesia bigemina* are the primary causative agents of bovine babesiosis in tropical and subtropical regions (Figueroa *et al*., 1998) [12]. Clinical signs of bovine babesiosis include fever, anemia, hemoglobinuria, ataxia, and, in severe cases, death. The parasites can often be detected in erythrocytes through direct microscopic examination (Bose *et al*., 1995; Bock *et al*., 2004) [6, 5].

In this study, the 18S rRNA gene was chosen as the target for molecular diagnosis of bovine babesiosis due to the availability of relevant sequence information in molecular databases. The sensitivity of LAMP was compared to conventional PCR using DNA samples from ten-fold serial dilutions of *B. bovis* and *B. bigemina*. The detection limits were: LAMP 1×10^{-5} and PCR 1×10^{-3}, demonstrating that LAMP is more sensitive than PCR for detecting low-level infections of *Babesia* spp. This highlights the potential of LAMP as a rapid and highly sensitive diagnostic tool for bovine babesiosis.

Babesia gibsoni **(Mandal *et al*., 2015)** [24]

Babesia gibsoni is a tick-borne blood protozoan parasite that causes babesiosis in dogs. The acute form of the disease is typically associated with remittent fever, progressive anemia, lethargy, thrombocytopenia, hemoglobinuria, and marked splenomegaly and hepatomegaly (Wozniak *et al*., 1997) [43]. However, chronic infections without apparent clinical signs are more common, making diagnosis at this stage particularly challenging (Irwin, 2010) [18].

In this study, a LAMP assay was developed and standardized for the detection of *B. gibsoni* in dogs, targeting the hypervariable region of the 18S rRNA gene. The assay demonstrated high sensitivity and specificity and was evaluated using field samples. Comparative results for detection in 75 field samples were: microscopy 23 positive, nested PCR 37 positive, and LAMP 43 positive.

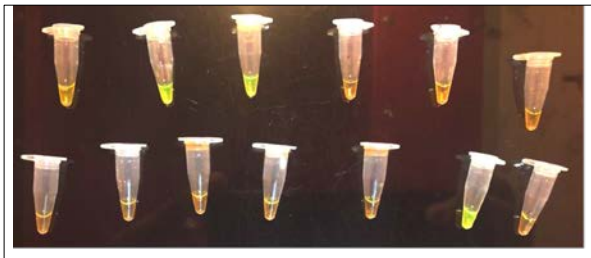
An advantage of the LAMP assay over conventional PCR is the ability to interpret results visually, as turbidity indicates a positive reaction, eliminating the need for gel electrophoresis and specialized equipment (Parida *et al*., 2008) [33]. This highlights LAMP as a rapid, sensitive, and field-applicable diagnostic tool for *B. gibsoni* infection in dogs.

Hepatozoon canis **(**Singh *et al*., 2020**)** [20]

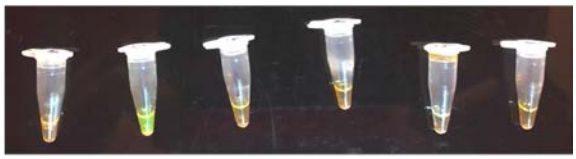
Canine hepatozoonosis, caused by *Hepatozoon canis*, is one of the most prevalent vector-borne infections in dogs worldwide, including India (Otranto and Dantas-Torres, 2010; Singh *et al*., 2017a) [37]. The parasite has a complex life cycle, with infection occurring through the ingestion of ixodid ticks harboring mature oocysts (Baneth *et al*., 2007) [4]. The primary vector of *H. canis* is the brown dog tick, *Rhipicephalus sanguineus sensu lato* (Nava *et al*., 2015) [28].

Clinical manifestations depend on parasitemia levels and the host immune response, ranging from asymptomatic infections to severe and potentially fatal disease (Gavazza *et al*., 2003) [14]. Typical clinical signs include fever, anorexia, lethargy, weight loss, lymphadenomegaly, and pale mucous membranes (Baneth and Weigler, 1997) [3].

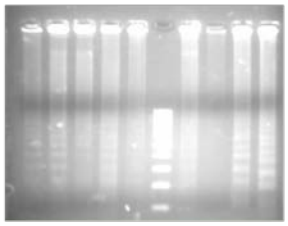
In this study, 250 blood samples from dogs were collected and analyzed using a LAMP assay optimized to target a portion of the 18S rRNA gene of *Hepatozoon* spp. The results of diagnostic testing were as follows: LAMP 75 positive, 18S PCR 28 positive, and microscopy 9 positive. The developed LAMP assay demonstrated high sensitivity and specificity, targeting only *H. canis* DNA, with no cross-amplification observed from other blood protozoan parasites, negative samples, or no-template controls. The assay produced characteristic ladder-like patterns specific to the *H. canis* target gene, confirming the absence of self-amplification. These findings regarding sensitivity and specificity are consistent with previous studies (Alhassan *et al*., 2007; He *et al*., 2009; Wang *et al*., 2010) [2, 15, 41]. The study highlights LAMP as a rapid, reliable, and specific diagnostic tool for detecting *H. canis* infection in dogs.



Field application of visual LAMP assay for detection of *H. canis*. Positives reactions: tube no. 2, 3, and 12. Negative reactions: tube no. 1, 4–11, and 13



Specificity of visual LAMP assay for detection of *H.canis*.
Tube 1: negative control.
Tube 2: *H. canis* DNA.
Tube 3: *B. vogeli* DNA.
Tube 4: *B. gibsoni* DNA.
Tube 5: *E. Canis* DNA.
Tube 6: *T. evansi* DNA

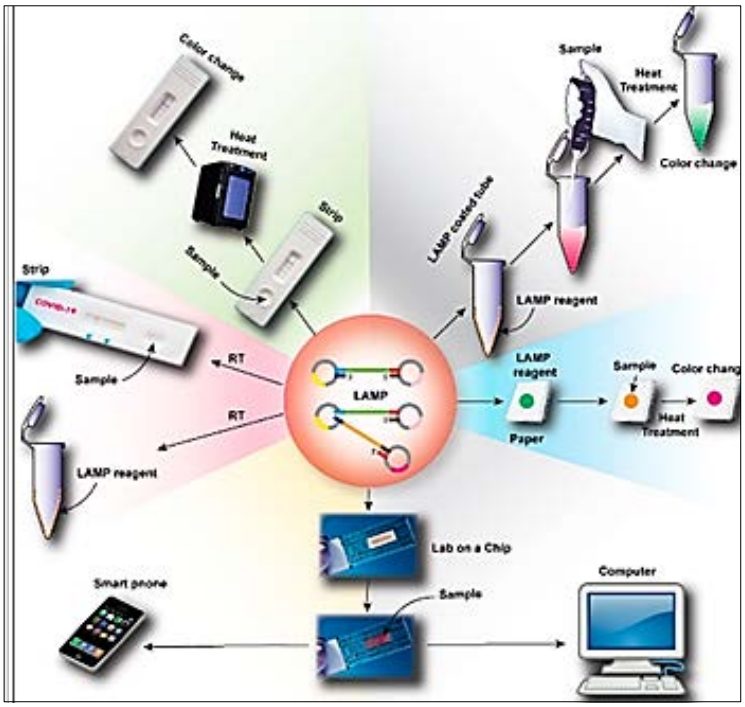


Field application of LAMP assay for detection of *H. canis*. Lane M: Generuler™ 100 bp Ladder. Lane P: positive control. Lane 7: negative control. Lane 1–6, 8: field samples

However, LAMP assay is very well suit for routine examination in field.

Different Forms of LAMP Assays

1. **Conventional LAMP:** Standard LAMP assay for DNA amplification under isothermal conditions.
2. **Reverse-Transcription LAMP (RT-LAMP):** Designed for RNA targets, where RNA is first reverse-transcribed to cDNA before amplification.
3. **Multiplex LAMP:** Simultaneous amplification of multiple target genes in a single reaction.
4. **Electric LAMP:** Utilizes electric fields or microfluidic platforms to enhance reaction efficiency and speed.
5. **In-Disc LAMP:** Miniaturized LAMP assay integrated on a disc or chip for portable, point-of-care diagnostics.
6. **Real-Time LAMP:** Allows continuous monitoring of amplification using fluorescence or turbidity, providing quantitative data in real time.



Different Forms of LAMP Assays

Conventional LAMP: Used for accurate molecular detection and differentiation of pathogens (Ziros *et al*., 2015) [45]. Promising for diagnostics, donor screening, epidemiological studies, and monitoring therapeutic interventions. Can be applied for field detection and early management of parasitic infections (Poole *et al*., 2015) [34].

Reverse-Transcription LAMP (RT-LAMP): A one-step nucleic acid amplification technique that converts RNA into complementary DNA. Highly effective in detecting pathogens with RNA genomes (Mori *et al*., 2013) [26]. Chander *et al*. (2014) [7] developed a novel RT-LAMP using OmniAmp polymerase, which is less inhibited by components in whole blood.

Multiplex LAMP (mLAMP): Designed for simultaneous detection of multiple target genes. Combines RT-LAMP and mLAMP in a single tube (Lau *et al*., 2015) [22]. All primer sets are added together to detect target organisms within 60 minutes. Colorimetric detection is performed using hydroxynaphthol blue (HNB) for naked-eye visualization of results. Uses primer sets for genes like SPECT2 and cytochrome oxidase subunit I, with real-time monitoring by turbidity meters and fluorescent image analysis.

Electric LAMP: Provides rapid and cost-effective testing of primer-target sequence compatibility. Enhances reaction efficiency and evaluates the potential of existing primers to detect newly discovered sequence variants (Salinas and Little, 2012) [36].

In-Disc LAMP (iD-LAMP): Allows estimation of amplification success or failure in a miniaturized, disc-based format.

Real-Time Colorimetric LAMP: Developed using miniaturized electronic components via 3D additive manufacturing and controlled by a smartphone application (Papadakis *et al*., 2020) [31]. Novel heating methods allow efficient DNA amplification with simultaneous visualization using a mini digital camera controlled by Raspberry Pi.

Designing Primers for LAMP Assay

Primer design is a critical step for the success of a LAMP assay. Specialized software is used to generate primer sets based on the target sequence, ensuring they meet the necessary design criteria for efficient amplification. The process typically involves selecting the target sequence file and specifying the desired parameters for primer construction.

Key specifications for LAMP primers include

1. **Primer Length:** Optimal length is generally 18-22 base pairs (bp), which balances specificity and efficient binding to the template at the annealing temperature.
2. **Melting Temperature (Tm):** The Tm is designed to be around 65 °C (64-66 °C) for F1c and B1c regions, about 60 °C (59-61 °C) for F2, B2, F3, and B3 regions, and around 65°C (64-66 °C) for loop primers.
3. **GC Content:** Primers should have a GC content of 40-65%, with 50-60% being ideal for stable and efficient primer-template binding.
4. **Secondary Structure:** Inner primers should avoid forming secondary structures, and 3’ ends of primers must not be complementary to prevent primer-dimer formation.
5. **Distance between Primers:** The amplified region between F2 and B2 should be 120-160 bases. The loop region between the 5’ ends of F2 and F1 should be 40-60 bases, while the distance between F2 and F3 should range from 0-60 bases.

Software Tools

- Primer Explorer Version 5 (http://primerexplorer.jp/lampv5e/index.html)
- Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/)

These tools ensure the design of highly specific and efficient primers for LAMP assays.

Advantages and Disadvantages of LAMP

Advantages: Loop-mediated isothermal amplification (LAMP) is highly valued for its sensitivity, specificity, low cost, simplicity, rapidity, and adaptability across diverse climatic conditions, with minimal instrumentation required (Mabey *et al*., 2004) [23]. The assay can be performed using a simple water bath or heating block that maintains isothermal conditions, making it suitable for field applications. Its specificity is exceptionally high (Tavare *et al*., 2011) [38], and the amplification efficiency is remarkable, with DNA amplified up to 10^9-10^{10} times within 15-60 minutes. Detection of LAMP products can be done visually using fluorescence dyes, real-time turbidity, or even by the naked eye (Tomita *et al*., 2008) [39]. Moreover, the method’s portability allows easy transport to diagnostic sites, facilitating rapid and on-site testing.

Disadvantages: Despite its advantages, LAMP has certain limitations. While highly sensitive and specific, in some cases it may be less sensitive than PCR (Aikawa *et al*., 2015) [1]. The technique is not suitable for cloning or other molecular biology applications (Sahoo *et al*., 2016) [35], and proper primer design is critical, often posing a significant constraint (Torres *et al*., 2011) [40]. Multiplexing in LAMP remains less efficient than in PCR (Dhama *et al*., 2014) [10]. The use of 4-6 primers targeting multiple regions increases the risk of primer-primer hybridization, potentially causing template-free amplification and false-positive results (Watts *et al*., 2014) [42]. Additionally, LAMP has a high risk of carryover contamination, which can produce false-positive results in negative controls (Karthik *et al*., 2014) [19]. The amplified products are highly stable and resistant to degradation, which, while advantageous for detection, can contribute to contamination issues (Fischbach *et al*., 2015) [13].

Conclusion

Loop-mediated isothermal amplification (LAMP) is an innovative and advanced gene amplification technique capable of amplifying target DNA with high sensitivity and specificity under isothermal conditions. It serves as a rapid and simple diagnostic tool for the prompt detection and identification of haemoprotozoan infections. LAMP offers an efficient platform for accurate and timely identification of various pathogens in both medical and veterinary fields.

Its simplicity, minimal equipment requirement, and ease of operation make it particularly valuable for use in developing countries. Overall, LAMP represents an ideal diagnostic method, meeting the criteria for an effective, affordable, and user-friendly test as recommended by the WHO.

Future Potential of LAMP

LAMP offers several significant advantages, including ease of handling, requiring only a simple water bath at a constant temperature, and rapid amplification of target DNA. The development of mobile biosensors incorporating LAMP technology could represent a major advancement in field diagnostics. Currently, LAMP is widely applied for the detection of diseases in humans, livestock, and plants. Its high specificity also makes it suitable for the early detection of genetic disorders. LAMP enables quick and accurate disease diagnosis, facilitating timely and effective treatment and management. One of the most innovative aspects of LAMP is the use of Bst DNA polymerase, which possesses strong strand displacement activity, enhancing the method's efficiency and reliability.

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